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Full Length Research Paper

Investigation of antioxidant capacity of *Melissa officinalis* L. essential oils

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Extraction of natural substances with antioxidant activity, to replace synthetic food preservatives has gained great importance. Medicinal plants have traditionally been used in folk medicine as well as to extend the shelf life of foods. *Melissa officinalis* L. an aromatic Lamiaceae has the main phenolic components that are useful to utilize as antioxidant. In this work, *M. officinalis* L. leaves was distilled in a Clevenger apparatus to obtain essential oil, chemical components was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Antioxidant activity of essential oil was evaluated by peroxide value, Iodine value and conjugated Dienes. Also the influence of this antioxidant on shelf life of Bezhi bersagh (a local confection in Ilam, Iran) was investigated. As a result, Neral and Beta-Caryophyllene was the most components in chemical analysis of essential oils. Free fatty acid during 1st till 8th week was increased significantly. Peroxide value increased linearly with storage time. Variation of both synthetic and natural antioxidant in aspect of Iodine values was reduces slowly. Conjugated Dienes (CD) changes in oil sample containing essential oil and butylated hydroxy-anisole (BHA) are same approximately. It was clear that shelf life of Bezhi Bersagh can be increased by adding antioxidants.

Key words: *Melissa officinalis*, antioxidant, GC-MS, peroxide value, iodine value.

INTRODUCTION

An antioxidant can be defined as “any substance that is in small quantities, normally in concentrations much lower than the oxidizable materials to be protected, is able to prevent or greatly delay its oxidation”. The antioxidants can be of synthetic or natural origin. The use of synthetic antioxidants is restricted in several countries, because of their undesirable long-term toxicological effects, including carcinogenicity (Gazzini et al., 1998) As a result, there is a great interest in finding antioxidants from natural sources for food Lipids containing polyunsaturated fatty acids are readily oxidized by molecular oxygen, and such oxidation proceeds by a free radical chain mechanism (Aruoma, 1998). The oil industry has to pay special attention in this context, as oils, fats and fatty foods suffer from stability problems (Wu and Nawar, 1986).

Traditionally, chemically synthesized compounds, such as butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT), are used as antioxidants in oil products. However, some of these compounds have been troubled for their safety (Bran, 1975, Whysner et al., 1994). The use of BHA and BTH is proved to be carcino-geneic. Therefore, there is an increasing interest in the antioxidative activity of natural compounds (Amakura et al., 2002). Aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods (Hulin et al., 2002). Most of their properties are due to essential oils produced by their secondary metabolism essential oils produced by their secondary metabolism (Adam et al., 1998). The extraction of natural substances with antioxidant activity, to replace synthetic food preservatives has gained great importance (Skerget et al., 2005). There are several methods for the evaluation of antioxidative action on fats and oils (Becker et al.,

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2004). Some methods are used for the assessment of early oxidative changes (unsaturated fatty acids, formation of free radicals and formation of primary oxidation products – peroxide value (POV)) and late oxidative changes (formation of secondary oxidation products – hydrocarbons). *Melissa officinalis* L. an aromatic Lamiaceaae is native to southern Europe and northern Africa, Caucasus and northern Iran (Meftahizade et al., 2010). Tinmaz et al. (2001) was reported that the highest essential oil ratio (0.14%) was obtained from the plants, cut in the beginning of blooming. The main components in the top third of plant are 39% citronellal, 33% citral (citronellol, linalool) and 2% geranial.

In this work, *M. officinalis* L harvested in the west-north of Iran was distilled in a Clevenger apparatus to obtain essential oil, which was analyzed by gas chromatography (GC) and gas Chromatography-mass spectrometry (GC-MS). The solid and liquid residues were solvent extracted to produce extracts, which were analyzed in terms of composition and antioxidant activity. The antioxidant activity was studied and some extracts were introduced into sunflower oil, being the oil samples analyzed by peroxide value and Iodine value to study the evolution of oil rancidity. Also the influence of this antioxidant on shelf life of Bezhi bersagh (a local confection in Ilam, Iran) was investigated. As well as the comparison between natural antioxidant with synthetic antioxidant (BHA) were done.

MATERIALS AND METHODS

M. officinalis L. aerial part was collected in August, 2009 in Uremia (West-North of Iran) and was air-dried. Before extraction, leaves were cut. The particle diameter after size reduction was estimated by using a sieving machine Retsch KS 1000 (Retsch, Haan, Germany), being the medium particle diameter calculated as 0.3 mm. Refined, bleached and deodorized (RBD) sunflower oil samples (supplied from Nazgol Factory, Kermanshah, Iran) were used to investigate the antioxidant activity of essential oil of *M. officinalis*. Sunflower oil was selected due to its high use in food as it is a rich source of linoleic acid and it can easily undergo rancidity due to high degree of unsaturation (Shahidi et al., 1992). The essential oils of *M. officinalis* were obtained by using a Clevenger apparatus. About 100 g of dried plant (leaves) were used and the distillation was conducted for 4 h. The amount of recovered oil was measured gravimetrically. Both the solid residue and the liquid residue of Clevenger distillation were used for preparation of extracts with eventually antioxidant activity by using the method explained by Ribeiro et al. (2001). The ground solid residues were boiled with distilled water for about 2/5 h, being then filtered through a coffee filter paper. The collected filtrate was acidulated with a solution of HCL 25% to a pH 2.5, followed by a filtration. The aqueous phase was mixed with diisopropilether (10:3) and allowed to separate. The upper anhydrous, aqueous layer was extracted two times. The organic phases were combined, dried with MgSO4 filtered, and evaporated using a rotary evaporator (Heidolph VV2000). Then the essential oils were analyzed by GC on a Hewlett-Packard 5890 Series II chromatograph equipped with a FID detector and a DB5 column (5% phenyl, 95% dimethyl polysil oxane), 0.32 mm id 50 m, film thickness 0.17 m. The column temperature was programmed to hold at 65°C for 15 min, then heated to 18°C at a slope of 2°C/ min, with a final isothermal hold at 180°C for 30 min. Injector and detector temperatures were 250 and 300°C, respectively. Carrier gas, N_2 was adjusted to a linear velocity of 2 ml/min. The samples were injected using the split mode (split ratio 1:20) being the injection volume 0.2 micro liter. The sample components were identified by comparing the retention times with those of chromatographic standard of the compounds (Sigma-Aldrich, Madrid, Spain). Peak areas were determined using a Hewlett-Packard 3396 Series II integrator. For quantitative analyses, the peak areas were converted to absolute values using response factors estimated from the standard compounds. Essential oils were also identified by GC-MS, by using a GC (shimadzu -9A) equipped with a DB-624 column (60 m x 0.32 mm id., film thickness 1.8 m), and linked to a MS detector (full scan), Carrier gas, He, was adjusted to a linear velocity of 1.5 ml/min. The injection volume was 1 micro liter. Determination of antioxidant activity in sunflower oils, storage of samples, seven refined, bleached and deodorized sunflower oil samples (SFO) were stored in triplicate in transparent polyethylene bottles of 250 ml capacity each. Out of total of twenty one bottles, seven bottles contained 120 ml blank deodorized, refined and bleached SFO (control). Other seven bottles contained 200 ppm of BHA per 120 ml deodorized, refined and bleached sunflower oil samples (SFO). Remaining seven bottles contained 200 ppm of essential oil per 120 ml of RBD sunflower oil (Duh and Yen, 1997).

Analysis of rancidity parameters in sunflower oil

Free fatty acid (FFA) values, peroxide values (PV) and iodine values (IV) were determined by following the recommended methods of AOCS (AOCS, 1989). Conjugated dienes (CD) were determined according to recommended methods of IUPAC (IUPAC, 1987). For the determination of CD sunflower oil samples were diluted with iso-octane to bring the absorbance within the limits. The absorbance was measured at wavelength 232 and 268 nm for CD and triene values respectively (Hitachi, U-o2001, Model 7400 spectrometer, Tokyo, Japan IUPAC, 1987). All these parameters were good indicators of lipid oxidation (Anwar et al., 2006; Gulcan et al., 2007). The PV was expressed as meq of oxygen / kg of fat, and was determined by the iodine titration method. Extracted oil samples (2 g) were weighed into test tubes. The oxidation of the potassium iodide, in acetic medium, by the active oxygen of the fat is followed by titration of the free iodine with sodium thiosulphate, using starch as indicator. The evolution of the peroxide value with storage time was studied. Primary oxidation processes in oil mainly form hydro peroxides, which are measured by the PV. In general, the lower the PV, the better the time quality of the oil. The IV ("iodine adsorption value" or "iodine number" or "iodine index") measures the number of reactive double bonds present in oil. A higher IV number indicates more double bonds in the sample and therefore that greater care will be needed to slow down oxidation, this were done according to methods of IUPAC, 1987. Finally an experiment was designed to evaluate the influence of synthetic (BHA, BHT) and natural antioxidant (*Melissa* essential oil) on maintenance and taste of Bezhi Bersagh during 9 weeks.

RESULTS AND DISCUSSION

Neral and Beta-Caryophyllene was the main components in chemical analysis of essential oils from *M. officinalis* L. harvested from Uremia, West-North of Iran (Table1). Results of a lot of researches have showed that essential oil of lemon balm and extracts (*M. officinalis* subsp. officinalis and of *M. officinalis* subsp. inodora) can be used as antioxidant (Marangui et al., 2004). Certainly

Table 1. Some components of essential oils obtained from *M. officinalis* leaves.

Components	Retention time	Mass percentage
Beta-ocimene Z	1020	0.2
Beta-ocimene E	1032	0.1
Citronellal	1086	0.01
Neral	1145	43.8
Geraniol	1221	5.3
Geranial	1246	5.2
Thymol	1258	7.9
Carvacrol	1274	0.8
Citronellyl formate	1276	0.2
Geranyl acetate	1362	2.3
Germacrene D	1375	0.3
Beta- caryophyllene	1424	13.5
Alpha-humulene	1448	0.7
Caryophyllene oxide	1575	0.3
Globulol	1581	6.8
Humulene epoxide	1617	0.3
5-cedranone	1629	0.2
Total		89.01

Table 2. Free fatty acid values in various RBD sunflower oil samples during 8 weeks.

No of weeks	Control samples	Essential oil sample	BHA sample
1st	0.065 ± 0.002	0.042 ± 0.008	0.045 ± 0.002
2nd	0.085 ± 0.003	0.050 ± 0.005	0.052 ± 0.006
3rd	0.095 ± 0.002	0.055 ± 0.006	0.056 ± 0.001
4th	0.121 ± 0.001	0.057 ± 0.004	0.056 ± 0.002
5th	0.148 ± 0.005	0.060 ± 0.001	0.062 ± 0.002
6th	0.192 ± 0.003	0.073 ± 0.006	0.075 ± 0.004
7th	0.252 ± 0.004	0.095 ± 0.002	0.098 ± 0.004
8th	0.273 ± 0008	0.099 ± 0.002	0.101 ± 0.004

Original value of control sample was 0.042 ± 0.002.

other population from different area has a various components. Rafat (2010) reported that there is a different between antioxidant capacity of different varieties and cultivars of same plant. Also, presence of phenolic compounds (Naral) is indicator of antioxidant activity. Several studies such as Tawaha et al. (2007), Othma et al. (2007) found good relation between content of phenolic compounds and the antioxidant activity in different plants. However, others study contradicted this relation; like Nsimba et al. (2008) reported that there are poor relation between antioxidant ability and phenolic compounds. Table 2 shows the changes in free fatty acid values, as can be seen, free fatty acid during 1st till 8th week were increased significantly in control samples (free from BHA or essential oil), but this increase is pretty low in BHA and essential oil. Also changes in oil contain BHA and essential oil was same approximately. This confirms the antioxidant ability of *M. Officianlis* essential oils. Gradually, due to hydrolysis of triglycerides, fatty acid will be released and this phenomenon will be accelerating by reaction of oil with moisture (Freja et al. 1999).

Changes in peroxide values have been shown in Table 3 in control samples. Peroxide value is one of the most widely used tests for oxidative rancidity in oils and fats. For this, oxidation degree on sunflower oil samples was determined by measuring POV in the absence and presence of antioxidants for 8 weeks. Results showed that peroxide value increased linearly with storage time. POV was 1.15 ± 0.02 in 1st week and immediately jumped to 7.35 ± 0.03 in 8th week; these changes were significant indicating the noticeable phenomenon of lipid oxidation. But in sample contains BHA/essential oil, this procedure is increased gradually, till was 0.95 ± 0.01 and 0.92 ± 0.04 in BHA and essential oil samples

Table 3. Peroxide values (meqkg^{-1}) of various RBD sunflower oil sample.

No of weeks	Control samples	Essential oil sample	BHA sample
1st	1.15 ± 0.02	0.45 ± 0.02	0.47 ± 0.04
2nd	2.25 ± 0.03	0.53 ± 0.01	0.55 ± 0.01
3rd	2.58 ± 0.04	0.60 ± 0.01	0.58 ± 0.05
4th	3.21 ± 0.03	0.62 ± 0.04	0.63 ± 0.04
5th	3.82 ± 0.05	0.73 ± 0.04	0.75 ± 0.04
6th	5.54 ± 0.04	0.85 ± 0.05	0.86 ± 0.05
7th	6.12 ± 0.05	0.88 ± 0.07	0.89 ± 0.01
8th	7.35 ± 0.03	0.92 ± 0.04	0.95 ± 0.01

Original value of control sample was 0.88 ± 0.004.

Table 4. Iodine values of various RBD sunflower oil sample.

No of weeks	Control samples	Essential oil sample	BHA sample
1st	131 ± 2.21	155 ± 2.25	153 ± 3.24
2nd	128 ± 1.52	152 ± 3.15	151 ± 2.35
3rd	127 ± 2.51	150 ± 3.24	149 ± 3.36
4th	118 ± 1.24	147 ± 2.5	148 ± 3.24
5th	110 ± 2.36	146 ± 1.25	146 ± 2.35
6th	108 ± 1.21	145 ± 2.13	145 ± 2.35
7th	107 ± 1.45	142 ± 2.34	143 ± 2.35
8th	106 ± 1.27	140 ± 2.36	140 ± 3.24

Original value of control sample was 135 ± 1.28.

Table 5. Conjugated Dienes in term of molar extinction co-efficient.

No of weeks	Control samples	Essential oil sample	BHA sample
1st	0.25 ± 0.02	0.15 ± 0.02	0.17 ± 0.02
2nd	0.27 ± 0.03	0.17 ± 0.01	0.21 ± 0.01
3rd	0.35 ± 0.04	0.19 ± 0.02	0.25 ± 0.03
4th	0.48 ± 0.04	0.22 ± 0.01	0.27 ± 0.03
5th	0.57 ± 0.07	0. 35 ± 0.01	0.32 ± 0.01
6th	0.65 ± 0.03	0.42 ± 0.02	0.44 ± 0.01
7th	0.75 ± 0.04	0. 47 ± 0.01	0.49 ± 0.02
8th	0.87 ± 0.04	0.58 ± 0.03	0.59 ± 0.04

Original value of control sample was 0.18 ± 0.02.

respectively.

Iodine value is a valid factor to monitor lipid oxidation (Naz et al., 2004). Table 4 and 5 shows the variation of both synthetic and natural antioxidant in point of view Iodine values, which in oil samples contain essential oil, after 8 week has arrived to 140 ± 2.36, also CD is the main measure in oil oxidation, and investigation of this variation is a authentic factor, which changes in oil sample containing essential oil and BHA are same approximately.

In the light of the above mentioned results and discussions, it is clear evidence that shelf life of Bezhi Bersagh can be increased by adding antioxidants (Table 6). Increase in lipid oxidation parameters for control samples was statistically significant. While these variations in Bezhi Bersagh containing BHA sample were statistically non significant. This measure can direct health benefits by decreasing the formation of reactive oxygen species in Bezhi Bersagh. The above-mentioned rancidity parameters are main indicators of deterioration of fats.

Table 6. Influence of essential oil on storage time of Bezhi Bersagh in room temperature.

Control sample (free essential oils)			Samples containing essential oils	
Storage time of Bezhi Bersagh in room temperature (week)	**pH**	**Taste variation**	**pH**	**Taste variation**
1st	6.1	------	6.1	------
2nd	6	------	6.1	------
3rd	5.9	Little	6.1	------
4th	5.7	Pretty sour	6	------
5th	5.5	sour	6	------
6th	5.2	Sour and acidous	5.9	Little sour
7th	3.9	Sour and acidous	6	Little sour
8th	3.9	Sour and acidous	6	Little sour
9th	3.8	Sour and acidous	5.5	Little sour

Conclusion

The result of the present study revealed that essential oils of *M. officinalis* L. has good potential for antioxidant activity and can be used in lipid containing foods. Its activity is comparable with synthetic antioxidant (BHA and BHT). And antioxidant activity is related to phenolic compounds like Citronellal and Neral. This applied research also showed that, utilize Melissa essential oils in delay decay of local confectionary that is, Bezhi Bersagh is useful and this fact can be added to other confection around the world.

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